The L55M polymorphism of paraoxonase-1 is a risk factor for rheumatoid arthritis

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ABSTRACT. Paraoxonase-1 (PON1) is a high-density lipoprotein-associated enzyme that exhibits antioxidant and antiatherogenic activities. We examined a possible association between T172A (L55M) and T(-107)C polymorphisms and rheumatoid arthritis. These polymorphisms were determined in 88 rheumatoid arthritis patients and 78 healthy subjects, using the tetra-amplification refractory mutation system-PCR method. The prevalence of the PON1 55MM genotype

was significantly greater among rheumatoid arthritis patients (17%) when compared to control subjects (5.2%) (odds ratio (OR) = 3.75; 95% confidence interval (CI) = 1.87-11.8, P = 0.025). In addition, the M allele was more frequent in rheumatoid arthritis patients (40%) than in healthy subjects (24.7%) (OR = 1.997; 95%CI = 1.243-3.210, P = 0.005). There were no significant differences in the -107C/T polymorphism in the promoter sequence of PON1 between rheumatoid arthritis and normal subjects (χ^2 = 0.861, P = 0.650). In conclusion, the PON1 55MM genotype is a risk factor for rheumatoid arthritis.

Key words: Rheumatoid arthritis; L55M polymorphism; PON1; Paraoxonase

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology affecting synovial membranes of multiple joints. Both genetic and environmental factors contribute to its etiopathogenesis (Deighton and Walker, 1991). It has been reported that reactive oxygen species (ROS) may play an important role in the pathogenesis of RA (Gambhir et al., 1997; Bauerova and Bezek, 1999). Under normal conditions, ROS are formed in oxidative processes at relatively low concentrations in all cells and tissues, and a variety of antioxidative mechanisms serve to control its production. Under pathological conditions, the levels of ROS are altered by increased production and/or inadequate removal, which results in oxidative stress, inducing cell damage and lipid peroxidation (Halliwell, 1994; Gambhir et al., 1997). Lipid peroxidation is a well-known mechanism of cellular damage in humans, and is used as an indicator of oxidative stress in cells and tissues.

Paraoxonase-1 (PON1) is coded by a gene located on chromosome 7q21.3-22.1 in a cluster with two similar genes, PON2 and PON3, whose physiological substrates are not yet recognized (Primo-Parmo et al., 1996). The 354-amino acid PON1 protein is exclusively bound to high-density lipoprotein. PON1 is recognized as an antioxidant enzyme as it hydrolyses lipid peroxides in oxidized lipoproteins (Blatter et al., 1993; Mackness et al., 1996; Aviram et al., 1998). PON1 also exerts paraoxonase and arylesterase activities, where it hydrolyzes organophosphates (paraoxon) and aromatic esters such as phenyl acetate (Gan et al., 1991).

It has been found that PON1 activity decreases in RA because of oxidative stress (Baskol et al., 2005; Isik et al., 2007). Paraoxonase activity is found to be comprehensively different among individuals, in part, as a result of the presence of polymorphisms. Two common coding region polymorphisms of PON1 (Q192R and L55M) lead to a change of both level and activity of the enzyme (Humbert et al., 1993; Garin et al., 1997; Mackness et al., 1998; Aviram et al., 2000). In addition, three polymorphic sites have been reported: T(-107)C, G(-824)A, and G(-907)C in the promoter region of PON1, which affects gene expression and serum concentrations, in particular the T(-107)C site (Leviev and James, 2000).

The data regarding PON1 Q192R polymorphisms in RA are controversial (Tanimoto et al., 2003; Hashemi et al., 2010). To the best of our knowledge there have been no reports investigating the PON1 T172A (L55M) and -107C/T polymorphisms in RA. We aimed to discover an association between T172A (L55M) and -107C/T polymorphism and RA.

MATERIAL AND METHODS

Patients

The study project was approved by the Ethics Committee of the Zahedan University of Medical Sciences and informed consent was taken from all participants. A total of 88 patients (80 women and 8 men) with an average age of 45.5 years (minimum 17, maximum 75) fulfilling American College of Rheumatology criteria for RA were examined (Arnett et al., 1988). The control group consisted of 78 healthy individuals (59 women and 19 men) with a mean age of 45.5 years (minimum 23, maximum 77), who were unrelated to RA patients. Blood samples were collected in Na-EDTA tubes from patients and healthy controls and stored at -20°C until DNA extraction.

DNA extraction

Two milliliters peripheral venous blood was collected from each subject for genomic DNA extraction as described previously (Hashemi et al., 2010).

Tetra-primer amplification refractory mutation system

Tetra-primer amplification refractory mutation system (ARMS) was designed for the detection of T172A (L55M) polymorphism and of -C107T PON1 according to the Ye et al. (2001) procedure. The primers used are shown in Table 1.

Table 1. Primers used for determination of -107C/T and PON1 L55M gene polymorphism.

Primers	-107C/T	L55M
Forward outer	5'-GCCAGTCCCATCCCCAAGAGGGTGAGCG-3'	5'-GGCTTTTGTACGTTTTGTG-3'
Reverse outer	5'-GAAAGTGCTGAGCTCCTGCGGTGGGGGC-3'	5'-CCGAAGAACACAAATATGCA-3'
Forward inner	5'-AGCGCCGATTGGCCCGCCACG-3'	5'-CAGAAACTGGCTCTGAAGTCA-3'
Reverse inner	5'-CTGCCGACCCGGCGGGGAGGTGT-3'	5'-TCCATTAGGCAGTATCTCGAA-3'

Polymerase chain reaction (PCR) was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, South Korea) according to the manufacturer recommended protocol. Into a 0.2-mL PCR tube containing the AccuPower PCR PreMix, 1 µL template DNA (~100 ng/µL), 1 µL of each primer (10 µM) and 15 µL DNase-free water were added. The total volume for the PCR was 20 µL.

For detection of L55M polymorphism, PCR cycling conditions were as follows: 5 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 59°C and 40 s at 72°C; 10 min at 72°C (Corbett Research, Australia). Each reaction was verified on a 2% agarose gel. Product sizes were: 262 bp for the A allele (allele M), 351 bp for the T allele (allele L), and 571 bp for the two outer primers (Figure 1A).

For determination of -107C/T polymorphism, PCR cycling parameters were 5 min at 95°C followed by 25 cycles of 20 s at 95°C, 20 s at 66°C, 15 s at 72°C and 10 min at 72°C (Corbett Research). Product sizes were: 116 bp for the C allele and 174 bp for the T allele, while the product size of the two outer primers was 246 bp.

The statistical analysis of the data was performed using the SPSS 17.0 software. Genotypes and alleles between groups were compared by the χ^2 test.
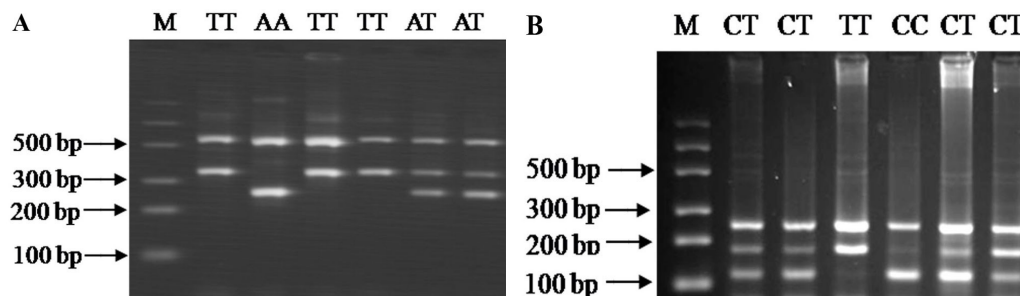
Figure 1. Results of tetra-ARMS-PCR of L55M (A) and (-107C/T) (B) of PON1 polymorphism. M = DNA marker.

RESULTS

PON1 T172A (L55M) polymorphism

The frequency of PON1 T172A (L55M) polymorphism in AR patients and normal subjects is shown in Table 2. The wild-type genotype (TT) was observed in 33/88 (37.5%) of the patients; whereas 40/88 (45.5%) were heterozygous (AT) and 15/88 (17%) were homozygous (AA). In the control group, the frequencies of genotypes were 43/77 (55.8%) for TT, 30/77 (39.0%) for AT and 4/77 (5.2%) for AA. There were significant differences regarding PON1 T172A polymorphisms among RA patients and normal subjects ($P < 0.05$).

Table 2. Genotype frequency of PON1 T172A (L55M) in rheumatoid arthritis (RA) patients and normal subjects.

Genotype	RA patients	Normal subjects	OR	95%CI	P
TT (LL)	33/38 (37.5%)	43/77 (55.8%)	0.516	0.279-0.956	0.043
AT (LM)	40/88 (45.5%)	30/77 (39.0%)	1.306	0.701-2.430	0.432
AA (MM)	15/88 (17.0%)	4/77 (5.2%)	3.750	1.870-11.84	0.025

OR = odds ratio; 95%CI = confidence interval at 95%.

As illustrated in Table 3, the prevalence of the 55M allele was significantly higher in RA patients (39.8%) than in healthy subjects (24.7%) (odds ratio = 1.997, 95% confidence interval = 1.243-3.210, P = 0.005), which illustrates that the 55M genotype is a risk factor for RA.

Table 3. Allele frequency of PON1 T172A (L55M) in rheumatoid arthritis (RA) patients and normal subjects.

Allele type	RA patients	Normal subjects	OR	95% CI	P
T allele (L allele)	106/176 (60.2%)	116/154 (75.3%)	0.491	0.308-0.797	0.005
A allele (M allele)	70/176 (39.8%)	38/154 (24.7%)	1.997	1.243-3.210	0.005

OR = odds ratio; 95%CI = confidence interval at 95%.

PON1 -107C/T polymorphism

The distribution of PON1 -107C/T polymorphism in AR patients and normal subjects is shown in Table 4. No significant differences were observed among AR and control subjects

regarding PON1 -107C/T polymorphism (χ^2 = 0.861, P = 0.650). The results indicated that there was no association between -107C/T PON1 polymorphism and AR.

Table 4. Distribution of PON1 -107C/T polymorphism in rheumatoid arthritis (RA) patients and normal subjects.

Genotype	RA patients	Normal subjects
CC	25/88 (28.4%)	20/75 (26.7%)
CT	41/88 (46.6%)	40/75 (53.3%)
TT	22/88 (25.0%)	15/75 (20.0%)

χ^2 = 0.861; P = 0.650.

There was no association between combined genotype polymorphism (55L/M + -170C/T) and AR (Table 5).

Table 5. Combined genotype polymorphism of PON1 in rheumatoid arthritis (RA) patients and normal subjects.

Genotype		Group	
55L/M	-170C/T	RA patients	Normal subjects
LL	CC	13 (14.8%)	16 (21.6%)
LL	CT	15 (17%)	17 (23%)
LL	TT	5 (5.7%)	8 (10.8%)
LM	CC	9 (10.2%)	3 (4.1%)
LM	CT	20 (22.7%)	20 (27%)
LM	TT	11 (12.5%)	6 (8.1%)
MM	CC	3 (3.4%)	1 (1.4%)
MM	CT	6 (6.8%)	2 (2.7%)
MM	TT	6 (6.8%)	1 (1.4%)

χ^2 = 11.04; P = 0.199.

DISCUSSION

Studies have demonstrated that PON1 gene polymorphisms cause a change from glutamine to arginine at the 192 position, leucine to methionine at the 55 position and T(-107) C in the promoter region of PON1, altering both level and activity of the enzyme (Humbert et al., 1993; Garin et al., 1997; Mackness et al., 1998; Aviram et al., 2000; Leviev and James, 2000). In the present study, we found significant differences between RA and control subjects regarding T172A (L55M) polymorphism. The frequency of the AA genotype (MM allele) was significantly higher in RA patients than in normal subjects. In addition the A allele (allele M) was detected more frequently in RA versus controls. No association was observed between T(-107)C single-nucleotide polymorphism in the promoter region of PON1 and RA.

In RA, increased ROS and production of lipid peroxidation, owing to synovial inflammation not only causes cellular damage, but also increases oxidative stress. In RA cases, increased oxidative stress and a decrease in antioxidants may have roles in both the pathogenesis of the disease (Nurcombe et al., 1991; Griffiths and Lunec, 1996) and the development of atherosclerosis (Sattar et al., 2003). Studies have shown that antioxidants are reduced in RA cases (McKeown et al., 1984; Jaswal et al., 2003; Isik et al., 2007). It has also been reported that there is an increase in the production of many cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-1 in the inflamed joints of RA cases (Ridderstad et al., 1991; Zwerina et al., 2005). Increased TNF-α increases the endogenous level of ROS production and contributes to oxidative stress (Miesel et al., 1996). Anti-inflammatory therapy with TNF-α

inhibitors has been shown to improve PON1 activity in RA patients (Popa et al., 2009).

Serum paraoxonase is an antioxidant enzyme because it hydrolyses lipid peroxides in oxidized lipoproteins (Mackness et al., 1996; Aviram et al., 1998). PON1 hydrolyses lipid peroxidation products and H_2O_2 (Feingold et al., 1998) and contributes to the prevention of low-density lipoprotein oxidation (Miesel et al., 1996; Mackness et al., 1998; Shih et al., 1998; Popa et al., 2009). An inverse association between PON1 activity and oxidative stress in serum and macrophages has been suggested (Rozenberg et al., 2003). It has been reported that serum PON1 activity decreased significantly in RA patients compared with healthy individuals (Maury et al., 1984; Tanimoto et al., 2003; Baskol et al., 2005).

There is some evidence showing an association between T172A (L55M) polymorphism and plasma PON1 activity. Garin et al. (1997) showed that the 55L allele (172T allele) has significantly higher concentrations of paraoxonase than the 55M allele (172A allele). Furthermore, Leviev et al. (1997) have found a significantly higher concentration of the L-type mRNA expression than the M-type in liver samples, supporting the idea that higher concentrations of serum paraoxonase are expressed in the L allele compared to the M allele.

In conclusion, we found that there is an association between PON1 L55M polymorphism and RA. No association was observed between T(-107)C single-nucleotide polymorphism in the promoter region of PON1 and RA. To the best of our knowledge, this study is important as it demonstrates the relationship of PON1 L55M polymorphisms and RA for the first time.

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